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Evaluation of the effects of exercise protocols on mitochondrial dysfunction in Alzheimer's disease

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Evaluation of the Effects of Exercise Protocols on Mitochondrial Dysfunction in Alzheimer's Disease

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Abstract
Alzheimer's disease is a debilitating disease that affects millions of people in the United States. This disease is caused by neurodegeneration and is the leading cause of dementia. There are not currently any medications that prevent and/or cure this disease. Recently, it has been thought that mitochondrial dysfunction may be the cause of this disease and it has been proposed that exercise may be a way to prevent this dysfunction. Currently, a comparison of different exercise protocols and the effects on mitochondrial dysfunction is not available. Because of this, an analysis of current literature has been performed to uncover the exercise protocols that may be most effective at combating mitochondrial dysfunction in the brain and skeletal muscle and therefore preventing and treating Alzheimer's disease.

Introduction
Alzheimer's disease (AD) is a brain disorder that inhibits memory and thinking skills, and eventually the ability to carry out simple tasks. Alzheimer's disease is the sixth ranked leading cause of death in the United States, and recent estimates indicate that the disorder may be the third leading cause of death for older people. During the preclinical stage of AD, abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain, and once healthy neurons lose function and connections with other neurons [8]. Although there are medications to treat symptoms associated with AD, there are no medications that cure or prevent the disease. Extensive research has shown that chronic exercise has the ability to enhance mood, cognition, cardiovascular efficiency, and many other processes throughout the body. Recently, studies have shown that exercise can upregulate certain transcription coactivators that may help in preventing and treating AD symptoms [9].

Although it has been shown that exercise can increase mood, cognition, and even aid in treating and preventing AD, exercise regimens that are specific for AD patients are not available. Exercise has many benefits when compared to medication. With the countless number of benefits that exercise provides to every system in the human body, it is an inexpensive way to improve one's health, with no real negative side effects, assuming that the individuals are healthy enough to exercise. Reviewing available literature may shed light on an exercise protocol that can best be utilized in the prevention and treatment of AD

Background
Over 5 million people live with Alzheimer's disease (AD) in the USA and a need for a treatment is becoming more and more critical as it is predicted that by the year 2025 there will be a 50% increase in patients with AD. Alzheimer's disease is the leading cause of dementia in aging populations, which is associated with neurodegeneration and neuronal loss. Patients with AD experience symptoms including cognitive alterations, memory loss and behavioral changes [1].

Alzheimer's disease is characterized by cognitive impairment and progressive neurodegeneration. The neurodegenerative process that is seen in AD is initially characterized by synaptic damage and neuronal loss. Synaptic loss is the leading

cause of cognitive impairment in patients with AD. Research shows that synaptic damage and neuronal loss in AD are related to progressive accumulation of beta amyloid oligomers [1].

Beta amyloid is a normal peptide generated throughout life, while amyloid plaques are a neuropathological hallmark of AD. Although amyloid precursor protein is one of the most studied proteins in science, its normal function remains unclear. Beta amyloid production and secretion is stimulated by synaptic activity, which is a unique and normal function of the nervous system, and generation of the small peptide is not inherently toxic. Amyloid plaques, however, represent an abnormal pathological lesion [2]. Electron microscopy analysis of postmortem brain shows that all forms of plaques are associated with neuropathology. Preplaque increased levels of beta amyloid correlate with AD-characteristic alterations in synapses [2].

Many lines of evidence support that beta amyloid peptides play an important role in Alzheimer's disease, which is the most common cause of dementia. While beta amyloid is generated from its precursor protein throughout life, the peptide is best known as the main component of amyloid plaques, the neuropathological hallmark of AD. Amyloid plaques are composed of aggregated beta amyloid, and neurofibrillary tangles are composed of microtubule-associated protein tau, and these are used as diagnostic criteria for AD. Neurofibrillary tangles, however, are less specific for AD and are seen in many neurodegenerative diseases. It has been shown that mutations in amyloid precursor protein (APP) and presenilin 1 and 2, which are proteases responsible for the cleavage of Aβ from APP, are both seen in familial forms of AD. Finally, biologic studies have shown that mutations in APP and presenilin proteases lead to higher amounts of disease-linked beta amyloid, all reasons pointing to beta amyloid as a major player in the pathogenesis of AD [2].

Decades of research indicate mitochondria from AD patients differ from those of non-AD individuals. Initial studies revealed structural differences, and subsequent studies showed functional deficits. Extensive research argues mitochondria may mediate, drive, and/or contribute to a variety of AD pathologies. The perceived significance of these mitochondrial changes continues to grow, and many currently believe AD mitochondrial dysfunction represents a reasonable therapeutic target [4].

Mitochondria are organelles that perform a number of roles in order to maintain cellular homeostasis and health. These organelles are best known for the role they play in synthesizing ATP through oxidative phosphorylation, but also play a role in fatty acid β-oxidation, phospholipid biosynthesis, calcium signaling, reactive oxygen species generation and apoptosis [30]. The requirement to sustain the mitochondrial population to ensure energy demands are met is central to cellular homeostasis. Many quality control mechanisms have evolved over time to ensure cellular homeostasis. Dysfunction of these mechanisms has become an emerging theme of many human diseases, including cancer, neurodegeneration, as well as aging [3].

Mutations have been hypothesized to contribute to aging due to the accumulation in mitochondrial DNA (mtDNA) over time. Mice expressing a proofreading-deficient version of the mtDNA polymerase accumulate mtDNA mutations and show features of accelerated aging. Increased oxidative stress markers are not seen with the accumulation of these mtDNA mutations, but accumulation of these mutations is correlated with the induction of apoptotic markers. The levels of apoptotic markers were also found to increase during aging in normal mice, thus, accumulation of mtDNA mutations that particularly promote apoptosis may be a central mechanism driving mammalian aging. The concept that DNA damage contributes to aging is supported by the finding that humans and mice carrying mutations in several genes involved in DNA repair display premature aging syndromes. It is likely that several types of DNA damage contribute to the aging process, and findings suggest that apoptosis and subsequent loss of irreplaceable cells may be an important mechanism of aging in mammals [4].

Mitochondrial dysfunction has been linked to AD through a hypothesis known as the mitochondrial cascade. This hypothesis states that mitochondrial function effects APP expression, APP processing, and beta amyloid accumulation in last-onset AD. The idea that mitochondrial dysfunction is linked to AD is known as the mitochondrial cascade and it consists of three parts. First, gene inheritance defines an individual's baseline mitochondrial function. Both mothers and fathers contribute to their offspring's AD risk, but because mitochondrial DNA is maternally inherited, mothers contribute more. Next, inherited and environmental factors determine the rate at which age-associated mitochondrial changes develop and manifest. If declining mitochondrial function drives aging phenotypes, then greater mitochondrial durability should associate with slower brain aging and lesser mitochondrial durability should associate with faster brain aging. Third, an individual's mitochondrial function and functional change rate influences their AD chronology. Those with low baseline function and fast rates of decline will develop symptoms and AD changes at younger ages than those with high baseline function and slow rates of mitochondrial decline. This hypothesis argues that if an amyloid cascade truly exists, mitochondrial function triggers it [4].

Debate continues over the origin of AD mitochondrial changes. Some argue amyloid-beta induces AD mitochondrial dysfunction. This view that does not challenge the amyloid cascade hypothesis and it may in fact help explain the hypothesis. Alternatively, data indicate mitochondrial dysfunction exists independent of amyloid-beta, potentially lies upstream of amyloid-beta deposition, and suggest a primary mitochondrial cascade hypothesis that assumes mitochondrial pathology supersedes amyloid-beta accumulation. Mitochondria, therefore, appear at least to mediate or possibly even initiate pathologic molecular cascades in AD [4].

Evidence has revealed that the regulation of mitochondrial turnover and function becomes impaired as a result of age in the brain and may contribute to neurodegeneration in AD. Inefficient cerebral metabolism and decreased expression and activity of mitochondrial enzymes important for metabolism is evident in affected brain regions where mitochondrial structure is altered [5]. Examples of

these mitochondrial alterations include intramedullary lesions, increased vacuoles, reduced cristae numbers, and an overall swollen shape [31]. AD brain mitochondria have reduced membrane potential, increased permeability, and produce excess reactive oxygen species (ROS), which damages proteins, lipids, and nucleic acids, and are believed to contribute to the pathogenesis seen in AD patients. Exercise training may be an effective strategy to delay mitochondrial aging and age-related dysfunction in humans by stimulating mitochondrial biogenesis and improving protein quality control. Skeletal muscle biopsies of humans performing high-intensity interval training showed an increase in skeletal muscle mitochondria and improved exercise performance. Biopsies performed in older men have shown that even with aging, exercise increases mtDNA and mitochondrial respiratory chain activity, which is likely related to increases in mitochondria biogenesis [5].

Mitochondrial alterations in structure and function have been shown in both AD patients and models, and it is thought that an imbalance in mitochondrial fusion and fission plays a part in mitochondrial dysfunction [31]. Mitochondria are dynamic organelles that undergo constant fission and fusion, which determines mitochondrial morphology, size, and also distribution and function of the organelles [32]. Recently, it has been shown that endurance exercise can balance the fusion and fission of mitochondria, which can improve mitochondrial function in the hippocampus of aging rats and slow cognitive deficits in AD models. [31].

It seems that increased physical activity, or even simply adopting active life style habits, may reduce the rate of mitochondrial decline due to aging. This exercise-induced increase in mitochondrial biogenesis is mediated through ROS as demonstrated by oral administration of antioxidants to rats, which impairs the exercise-induced increase in mitochondrial mRNA and protein levels. Importantly, it has been found that exercise training increases mitochondrial biogenesis through involvement of mtDNA, PGC-1α, and various other proteins in the brain stem, cortex, frontal lobe, hippocampus, hypothalamus, and midbrain, and this may have important implications with respect to AD and other age-related diseases, which are characterized by mitochondrial dysfunction. Therefore, exercise could be a promising option for reducing the negative effects of aging and therefore decrease the risk of AD [5].

Peroxisome proliferator-activated receptor ϒ co-activator 1α (PGC-1α) plays an important role in promoting mitochondrial biogenesis in response to exercise training. Downregulation of PGC-1α has been shown to contribute to mitochondrial deterioration [7]. PGC-1α activates the mitochondrial transcription factor A (TFAM), which is responsible for transcribing nuclear encoded mitochondrial proteins including structural proteins and proteins involved in mitochondrial DNA transcription, translation, and repair [10]. Because of the positive role that PGC-1α plays in mediating mitochondrial function, exercise protocols that upregulate the protein should be thought of as effective means of maintaining proper mitochondrial function.

Research has shown that PGC-1α is expressed in tissues with a high-energy demand, such as skeletal muscle, but fewer studies have studied the role of PGC-1α in regards to mitochondrial dysfunction in AD. Impairment of PGC-1α in the brain triggers the degeneration of neurons in the brain by inducing mitochondrial dysfunction and PGC-1α also plays a vital role in the detoxification of reactive oxygen species. This evidence supports the idea that PGC-1α plays a vital role in both skeletal muscle and the brain. Skeletal muscle studies were included in this review because of the comparable effects that exercise has on each [33].

Methods

An extensive review of available literature through the PubMed database was performed to identify articles that evaluated the effects of different forms of exercise on mitochondrial function in the brain and skeletal muscle. Articles that were targeted were ones that specifically focused on the effects of exercise on PGC-1α and oxidative capacity. These articles were specifically targeted because of the role that PGC-1α plays in mediating mitochondrial function. A core problem in the brains of AD patients is oxidative stress and PGC-1α controls the expression of genes that are related to the production of reactive oxygen species. Along with this, impairment of PGC-1α has been shown to trigger mitochondrial dysfunction and neuronal degradation [34]. Taking these factors into consideration, PGC-1α seemed to be a good target when studying mitochondrial function. Studies that did not observed the effects of exercise on PGC-1α and its regulatory proteins were excluded from this study.

After accumulating relevant articles, the articles were added to a master table (see appendix A). The articles were organized according to title of the study, overall outcome, exercise protocol, and participant information. The range of subjects used was very broad and included young and old humans, AD patients, and various animal models. With the exception of one outlier, all articles have been published within the past two decades. To distinguish between the effects of exercise on skeletal muscle and the brain, the master table was divided into tables 1 and 2, respectively. The information was divided in this fashion to display how exercise has the ability to upregulate PGC-1α in different brain regions, as well as in skeletal muscle. The information from Table 1 was organized by specific exercise protocol, and Graph 1 and 2 were constructed using this information to better display the effects of different forms of exercise on PGC-1α expression in skeletal muscle.

Graph 3 was constructed by using information from Table 1. Eight studies from Table 1 that studied the effects of endurance exercise on PGC-1α in skeletal muscle were used to show how the duration of exercise can have an effect on PGC-1α expression.

Results

Training protocols and participant characteristics for every study are shown in Appendix A. A range of different training protocols was used including resistance training (n=4), endurance training (n=19), and a combination of both (n=1). Studies

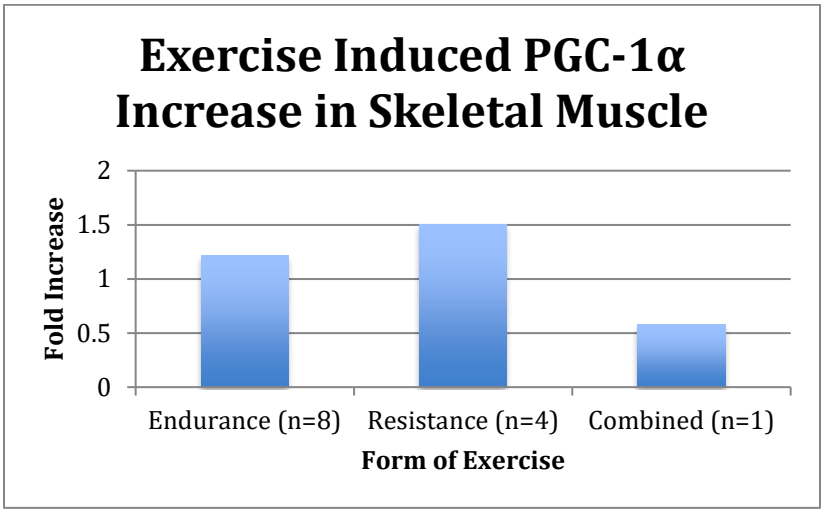
were further divided into skeletal muscle studies (Table 1) and brain studies (Table 2).

Table 1: Skeletal Muscle Exercise Studies

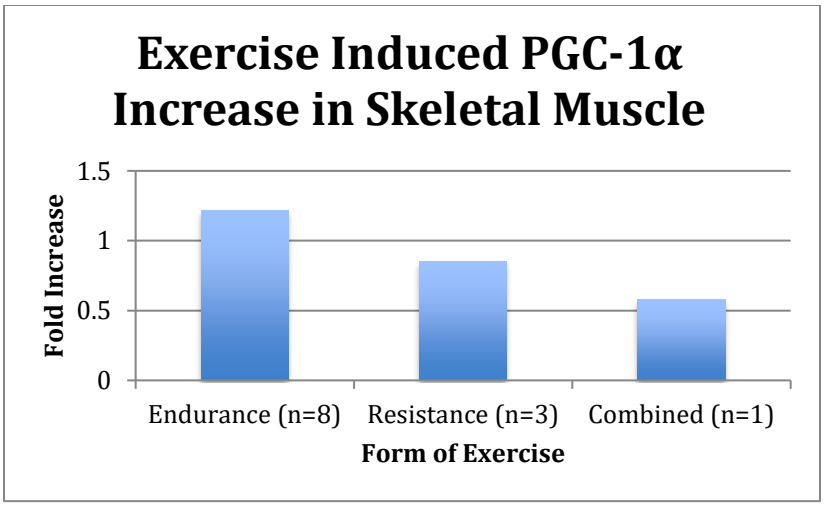
Study	Effect	Mode of Exercise	Subject	Frequency, Intensity, and Duration
Meshikova, E.	53 +/- 15% increase in mtDNA Activity of NADH oxidase doubled.	Treadmill, stationary bicycles, or outdoor walking	8 elderly volunteers (67.3 +/- 0.6 yr), 3 women 5 men	4-6 exercise sessions weekly for 12 weeks Exercise increased from low to moderate intensity.
Koltai, E.	PGC-1α levels were equal in old and young exercise groups (1-fold ↑)	Treadmill	24 male Wistar rats (12 3 month old 12 26 month old)	30 minutes per day for 6 weeks Young: 10 m/min, increasing to 22 m/min, Old: 10 m/min increasing to 13 m/min,
Johnson, M.	YS vs. OS: 625 genes differentially expressed. YT vs. OT: 1287 genes differentially expressed. MAPK transcription levels ↑	Cycling and running	10 young sedentary 10 old sedentary 10 young trained 10 old trained	6 days per week over past 4 years
Irving, B.	ET: ↑ in PGC-1α 44% in young and 15% in old RT: ↑ 80% in young, 36% in old CT: ↑ 25% in young, 58% in old	ET: Cycling RT: Compound Lifts CT: Combination	34 young (18-30) and 31 old (>65)	ET: 1 hour, 5 days/week for 8 weeks RT: 4 sets of 8-10 reps, 4 days/week for 8 weeks CT: 30 min 5 days/week and 2/3 the RT volume 4 days/week Moderate intensity
Safdar, A.	PGC-1α protein content ↑ (2.4-fold)	Treadmill	36 3 month old mice	15m/min. for 90 min.
Little, J.	Protein content of PGC-1α ↑~24% Whole muscle PGC-1α was unchanged.	Cycling	7 healthy men (21 +/- 1 yr)	6 sessions over 2 weeks 8 -12 60s high intensity intervals
Wang, L.	E: PGC-1α ↑ 1-fold ER: PGC-1α ↑ 1.75-fold	Cycling Leg press	7 men, 2 women (26 +/- 1.2)	Moderate intensity cycling Moderate intensity leg press
Holloszy, J.	ETC Enzymes doubled Cytochrome C increased by 2-fold.	Treadmill	Male Wistar Rats	Moderate exercise 2x/day, 4 days/week, increasing to high intensity sprints
Silvennoinen, M.	EE: PGC-1α ↑ 1.8-fold RE: PGC-1α ↑ 4-fold	Bilateral leg press Treadmill	RE: 11 healthy males EE: 8 Healthy males	Moderate intensity leg press Moderate-high intensity treadmill
Kang, C.	OT PGC-1α = 35% greater than young. No difference seen between OT and Y rats OT: 2.3-fold ↑ in PGC-1α compared to sedentary	Treadmill	Male Fisher 344 x Brown Norway F_1 hybrid rats, 4 months and 22 months	Trained: Moderate intensity Sedentary: Low intensity
Schwarz, NA.	No ↑in PGC-1α mRNA	Lower body resistance exercise	Ten healthy men (23.7 +/- 2.8 years)	Moderate and high intensity
Short, KR.	↑ in PGC-1α, 55%; NRF-1, 15%; TFAM, 85%	Stationary bicycle	Forty-nine women and 41 men between the ages of 21-87 years	Stationary bicycle: 3x/week moderate intensity increasing to 4x/week at high intensity

Table 1 illustrates the effects of exercise in skeletal muscle.

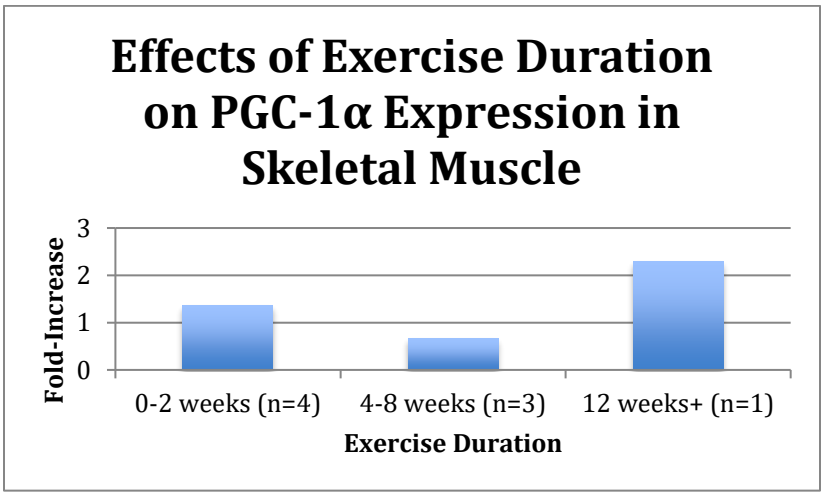
The results of different forms of exercise in skeletal muscle are shown in Table 1. Graphs 1, 2, and 3 were created to better illustrate the effects of exercise on PGC-1α.



Graph 1 illustrates the effect of different forms of exercise on PGC-1α expression.



Graph 2 illustrates the effect of different forms of exercise on PGC-1α expression.



Graph 3 illustrates the effects of different durations of exercise on PGC-1α expression.

Graph 1 illustrates the effects of different forms of exercise on PGC-1α expression. This particular graph makes it appear that resistance exercise is the most effective

at upregulating PGC-1α, however one study [19] showed an abnormally high increase in PGC-1α through resistance exercise (4-fold increase). This outlier was removed from the results and Graph 2 was created to better illustrate how different forms of exercise affect PGC-1α expression. Endurance exercise was most effective at upregulating PGC-1α, with an average increase of 1.2-fold. Resistance training appeared to be the second most effective form of exercise, with an average increase of 0.9-fold. Finally, it appeared that a combined training regimen is least effective at upregulating PGC-1α, however because of there only being a single combined exercise study, more research is required.

Graph 3 illustrates how the duration of endurance exercise plays a role in affecting PGC-1α expression in skeletal muscle. Because there is only one study that exercised participants for 12+ weeks, it is hard to definitively propose that 12+ week exercise protocols are most effective at upregulating PGC-1α, however these results do agree with current hypotheses.

Table 2: Brain Tissue Exercise Studies

Study	Effect	Mode of Exercise	Subject	Frequency, Intensity, and Duration
Steiner, J.	SIRT1 mRNA ↑ 2-fold in the CX, FL, HY, HC, and MB, but not in the BS or CB. PGC-1α ↑ up to 3-fold in the BS, CX, FL, HC, HY, MB, and CB	Treadmill	Male ICR mice. 16-19 exercise and sedentary	Eight weeks of treadmill running 1 hr/day, 6 days/week at 25 m/min. and 5% incline.
Zhang, Q.	PGC-1α protein levels ↑ 7 days of training was required for protein levels of NRF-1 and TFAM to increase	Treadmill	26 healthy male Sprague-Dawley rats	Exercised on rat treadmill. 7 days at 30 min per day. Low intensity
Lezi, E.	↑of nuclear PGC-1α levels 50% Mitochondrial DNA copy number ↑	Six-lane treadmill	24 C57BL/6 male mice, 18 months old	Exercise: 8 weeks, 5 days per week, 2 sessions per day, Moderate-high intensity
Azimi, M.	Exercise prevented suppression of PGC-1α ↑ in mRNA levels of PGC-1α (58%) in the hippocampus	Treadmill	70 adult male Winstar rats	5 days/week for 4 weeks First and second week Moderate-high intensity
Lezi, M.	Liver PGC-1α ↑ but liver and brain PGC-1α protein levels were unchanged.	Treadmill	Twelve 4 month old C57BL/6 mice	2x/day, 5 days/week for six weeks Moderate exercise
Marques-Alexio, I.	PGC-1α and TFAM ↑ 50% in the brain cortex. No ↑ in the cerebellum	Treadmill	18 male Sprague-Dawley rats	Rats exercised for 60 min/day 5 days/week for 12 weeks Moderate-high intensity
Bayod, S.	PGC-1α levels ↑ in cortex No hippocampal ↑	Treadmill	29 Sprague-Dawley rats	4-5 days/week for 36 weeks Low intensity

Table 2 illustrates the effects of endurance exercise in the brain.

Of the 7 studies that examined the effects of endurance exercise in the brain, 6 showed increases in PGC-1α. The results showed a 1-fold increase on average in these animals, which was fairly comparable to the results seen in skeletal muscle. One 36-week low intensity exercise study [29] showed increases in PGC-1α in the

cortex, but no significant increase in the hippocampus. A different 4-week exercise study that utilized high intensity exercise [26] reported a 58% increase in PGC-1α levels in the hippocampus. These results could suggest that PGC-1α expression is only upregulated in certain regions of the brain when high intensity exercise is performed.

Discussion

Exercise training has been shown to be an effective means of enhancing mitochondrial function and efficiency. Of the 12 skeletal muscle studies, 11 showed that exercise increases PGC-1α and oxidative capacity. When comparing endurance training to resistance training, endurance training seems to be more efficient in terms of increasing mitochondrial efficiency. Graph 1 illustrates the average increase in PGC-1α with each form of exercise and the results for endurance and resistance exercises are very similar. This similarity, however, is somewhat misleading. Because of the small amount of resistance based exercise regimens, the result of one study showing a four-fold increase in PGC-1α due to resistance exercise makes this form of exercise appear more effective at upregulating this protein than it is shown in other studies.

There are several reasons that the effects of exercise on PGC-1α were studied in skeletal muscle and the brain. First, as I said there were a very limited number of articles that observed the effects of exercise on PGC-1α in the brain. Next, The effects of exercise on PGC-1α in the brain and skeletal muscle have comparable results, suggesting that exercise upregulates PGC-1α in skeletal muscle and the brain in a similar fashion. Finally, the only biopsies performed on brain tissue post-exercise are performed on animal models, specifically rodents, so rodents are the only subjects used when examining the effect of exercise on PGC-1α in the brain. The beauty of exercise-based therapies is that human studies can be readily performed, assuming the subjects are healthy enough to exercise. Exercise is an inexpensive way to treat different ailments without the adverse side effects that can be seen in various pharmaceuticals. Although the animal models are very important when examining brain PGC-1α, human models were most important when studying such effects, and this is why skeletal muscle studies were included in this analysis.

Another important factor when designing an exercise protocol is the duration of that protocol. Graph 2 was constructed to compare the effect of different durations of endurance protocols on skeletal muscle PGC-1α. At first sight the results can again be somewhat misleading. The 0-2 week protocols appear more effective than the 4-8 week protocols, however, this is likely due to the fact that when the biopsies are performed on the 0-2 week models, the PGC-1α levels are still spiked from the exercise that was just performed. This in turn leads to the inflated results of the 0-2 week studies.

It seems that exercise may be a promising option for upregulating PGC-1α and preventing mitochondrial dysfunction. The prevention of mitochondrial dysfunction may prove to be a means of preventing and treating Alzheimer's disease, but much more research must be done in order to confirm this hypothesis. In order to find the

best way to treat AD through exercise regimens, many different forms of exercise should be studied. At this time it seems that chronic endurance training is more effective in comparison to resistance training, however after more research it would not be surprising to see a combined exercise regimen be the most effective method for preventing and treating AD.

Although exercise has not been shown to cure AD, many studies have shown that it has positive effects on both cognition and motor function. Avoiding a sedentary lifestyle and practicing life long exercise is vital to a healthy life. Life long exercise has the ability to maintain proper mitochondrial function and possibly the ability to prevent many diseases, including AD. Although exercise is becoming a promising means of preventing and treating AD, the benefits of an active lifestyle go far beyond the prevention of a single disease.

With the literature available, adopting an endurance based exercise regimen is an effective way to increase PGC-1α, prevent mitochondrial dysfunction, and maintain a healthy lifestyle, free of neurodegeneration. The seemingly infinite number of benefits that exercise provides is just beginning to be uncovered and more research must be done in order to uncover these benefits. The effects of different modes and combinations of exercises need to be studied in order to truly understand the most efficient means of preventing and treating Alzheimer's disease.

References

1. Crews, L., & Masliah, E. (2010). Molecular mechanisms of neurodegeneration in Alzheimer's disease. *Human molecular genetics*, *19*(R1), R12–R20. doi:10.1093/hmg/ddq160

2. Gouras, G. K., Olsson, T. T., & Hansson, O. (2014). β-Amyloid peptides and amyloid plaques in Alzheimer's disease. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*, *12*(1), 3-11.

3. Kotiadis, V. N., Duchen, M. R., & Osellame, L. D. (2014). Mitochondrial quality control and communications with the nucleus are important in maintaining mitochondrial function and cell health. *Biochimica et biophysica acta*, *1840*(4), 1254-65.

4. Swerdlow R. H. (2018). Mitochondria and Mitochondrial Cascades in Alzheimer's Disease. *Journal of Alzheimer's disease : JAD*, *62*(3), 1403-1416.

5. Onyango, I. G., Dennis, J., & Khan, S. M. (2016). Mitochondrial Dysfunction in Alzheimer's Disease and the Rationale for Bioenergetics Based Therapies. *Aging and disease*, *7*(2), 201-14. doi:10.14336/AD.2015.1007

6. Metaxas, A., & Kempf, S. J. (2016). Neurofibrillary tangles in Alzheimer's disease: elucidation of the molecular mechanism by immunohistochemistry and tau protein phosphor-proteomics. *Neural regeneration research*, *11*(10), 1579-1581.

7. Kang C, Chung E, Diffee G, Ji LL. Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1alpha. Exp. Gerontol. 2013;48:1343–1350. doi: 10.1016/j.exger.2013.08.004.

8. (2016). Alzheimer’s Disease Fact Sheet*. National Institute on Aging.*

9. Basso, J. C., & Suzuki, W. A. (2017). The Effects of Acute Exercise on Mood, Cognition, Neurophysiology, and Neurochemical Pathways: A Review. *Brain plasticity (Amsterdam, Netherlands)*, *2*(2), 127-152. doi:10.3233/BPL-160040

10. Li, X., Wang, H., Gao, Y., Li, L., Tang, C., Wen, G., Yang, Y., Zhuang, Z., Zhou, M., Mao, L., … Fan, Y. (2016). Quercetin induces mitochondrial biogenesis in experimental traumatic brain injury via the PGC-1α signaling pathway. *American journal of translational research*, *8*(8), 3558-66.

11. Menshikova, E. V., Ritov, V. B., Fairfull, L., Ferrell, R. E., Kelley, D. E., & Goodpaster, B. H. (2006). Effects of exercise on mitochondrial content and function in aging human skeletal muscle. *The journals of gerontology. Series A, Biological sciences and medical sciences*, *61*(6), 534–540.

12. Koltai, E., Hart, N., Taylor, A. W., Goto, S., Ngo, J. K., Davies, K. J., & Radak, Z. (2012). Age-associated declines in mitochondrial biogenesis and protein quality control factors are minimized by exercise training. *American journal of physiology. Regulatory, integrative and comparative physiology*, *303*(2), R127–R134. doi:10.1152/ajpregu.00337.2011

13. Johnson, M. L., Lanza, I. R., Short, D. K., Asmann, Y. W., & Nair, K. S. (2014). Chronically endurance-trained individuals preserve skeletal muscle mitochondrial gene expression with age but differences within age groups remain. *Physiological reports*, *2*(12), e12239. doi:10.14814/phy2.12239

14. Irving B. A., Lanza I. R., Henderson G. C., Rao R. R., Spiegelman B. M., Nair K. S. (2015). Combined training enhances skeletal muscle mitochondrial oxidative capacity independent of age. *J. Clin. Endocrinol. Metab.* 100 1654–1663. 10.1210/jc.2014-3081

15. Safdar, A., Little, J. P., Stokl, A. J., Hettinga, B. P., Akhtar, M., & Tarnopolsky, M. A. (2011). Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *The Journal of biological chemistry*, *286*(12), 10605–10617. doi:10.1074/jbc.M110.211466

16. Little J. P., Safdar A. S., Wilkin G. P., Tarnopolsky M. A., and Gibala M. J.. 2010. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. J. Physiol. 588:1011–1022.

17. Wang L., Mascher H., Psilander N., Blomstrand E., Sahlin K. wang. *Journal of Applied Physiology*. 2011;111(5):1335–1344. doi: 10.1152/japplphysiol.00086.2011.

18. Holloszy J. O. 1967. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J. Biol. Chem.242:2278–2282.

19. Silvennoinen, M., Ahtiainen, J. P., Hulmi, J. J., Pekkala, S., Taipale, R. S., Nindl, B. C., … Kainulainen, H. (2015). PGC-1 isoforms and their target genes are expressed differently in human skeletal muscle following resistance and endurance exercise. *Physiological reports*, *3*(10), e12563. doi:10.14814/phy2.12563

20. Steiner J. L., Murphy E. A., McClellan J. L., Carmichael M. D., Davis J. M. Exercise training increases mitochondrial biogenesis in the brain. *Journal of Applied Physiology*. 2011;111(4):1066–1071. doi: 10.1152/japplphysiol.00343.2011.

21. Zhang Q., Wu Y., Zhang P., Sha H., Jia J., Hu Y. Exercise induces mitochondrial biogenesis after brain ischemia in rats. Neuroscience. 2012;205:10–17. doi: 10.1016/j.neuroscience.2011.12.053.

22. Kang C, Chung E, Diffee G, Ji LL. Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1alpha. Exp. Gerontol. 2013;48:1343–1350. doi: 10.1016/j.exger.2013.08.004.

23. Lezi E, Burns JM & Swerdlow RH Effect of high-intensity exercise on aged mouse brain mitochondria, neurogenesis, and inflammation. Neurobiol. Aging 35, 2574–2583 (2014).

24. Schwarz NA, McKinley-Barnard SK, Spillane MB, Andre TL, Gann JJ, Willoughby DS. Effect of resistance exercise intensity on the expression of PGC-1α isoforms and the anabolic and catabolic signaling mediators, IGF-1 and myostatin, in human skeletal muscle. Appl Physiol Nutr Metabol. (2016) 41:856–63. 10.1139/apnm-2016-0047

25. Short KR, Vittone JL, Bigelow ML, Proctor DN, Rizza RA, Coenen-Schimke JM, et al. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes. 2003;52:1888–1896. doi: 10.2337/diabetes.52.8.1888.

26. Azimi M., Gharakhanlou R., Naghdi N., Khodadadi D., Heysieattalab S. Moderate treadmill exercise ameliorates amyloid-β-induced learning and memory impairment, possibly via increasing AMPK activity and up-regulation of the PGC-1α/FNDC5/BDNF pathway. *Peptides*. 2018;102:78–88. doi: 10.1016/j.peptides.2017.12.027.

27. Lezi E, Lu J, Burns JM, Swerdlow RH. Effect of exercise on mouse liver and brain bioenergetic infrastructures. Exp Physiol. 2013;98:207–219. doi: 10.1113/expphysiol.2012.066688.

28. Marques-Aleixo I, Santos-Alves E, Balça MM, Rizo-Roca D, Moreira PI, Oliveira PJ, Magalhães J, Ascensão A. Physical exercise improves brain cortex and cerebellum mitochondrial bioenergetics and alters apoptotic, dynamic and auto(mito)phagy markers. Neuroscience. 2015;301:480–495. doi: 10.1016/j.neuroscience.2015.06.027.

29. Bayod S., Del Valle J., Canudas A. M., Lalanza J. F., Sanchez-Roige S., Camins A., et al. . (2011). Long-term treadmill exercise induces neuroprotective molecular changes in rat brain. J. Appl. Physiol. 111, 1380–1390. 10.1152/japplphysiol.00425.2011

30. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, et al. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science. 2005;309(5733):481–4. 10.1126/science.1112125

31. Yan, Qing-Wei, Na Zhao, Jie Xia, Bai-Xia Li, and Ling-Yu Yin. "Effects of Treadmill Exercise on Mitochondrial Fusion and Fission in the Hippocampus of APP/PS1 Mice." *Neuroscience Letters.* 701: 84-91. Web.

32. Wang, X., Su, B., Lee, H. G., Li, X., Perry, G., Smith, M. A., & Zhu, X. (2009). Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(28), 9090–9103. doi:10.1523/JNEUROSCI.1357-09.2009

33. Sweeney, G., & Song, J. (2016). The association between PGC-1α and Alzheimer's disease. *Anatomy & cell biology*, *49*(1), 1–6. doi:10.5115/acb.2016.49.1.1

34. Du, X., Wang, X., & Geng, M. (2018). Alzheimer's disease hypothesis and related therapies. *Translational neurodegeneration*, *7*, 2. doi:10.1186/s40035-018-0107-y

Appendix A

Study	Effect	Mode of Exercise	Subject	Frequency, Intensity, and Duration
Effects of Exercise on Mitochondrial Content and Function in Aging Human Skeletal Muscle [11]	There was a 53 +/- 15% increase in mtDNA content with training. Activity of NADH oxidase was approximately doubled. Succinate oxidase increased by 62 +/- 13%.	Treadmill, stationary bicycles, or outdoor walking	8 elderly volunteers (67.3 +/- 0.6 yr), 3 women 5 men	4-6 exercise sessions weekly 12 weeks First 4 weeks: 30 min. at low intensity Next 4 weeks: 40 min. at same intensity Last 4 weeks: At least 40 min. at moderate intensity
Age-Associated Declines in Mitochondrial Biogenesis and Protein Quality Control Factors are Minimized by Exercise Training [12]	There are significant reductions in PGC-1α levels with age, which the study showed to be prevented by exercise training. PGC-1α levels of young exercised rats were roughly equal to those of old exercised rats (1-fold increase).	Treadmill	24 male Wistar rats (12 3 month old 12 26 month old)	Young: 10 m/min, 5% incline, gradually increasing to 22 m/min, 10% incline Old: 10 m/min, 5% incline, gradually increasing to 13 m/min, 10% incline
Chronically Endurance-Trained Individuals Preserve Skeletal Muscle Mitochondrial Gene Expression with Age but Differences Within Age Groups Remain [13]	YS vs. OS: 625 genes differentially expressed. YT vs. OT: 1287 genes differentially expressed. Genes involved in mitochondrial oxidative phosphorylation were higher in trained participants independent of age.	Cycling and running	10 young sedentary 10 old sedentary 10 young trained 10 old trained	Sedentary participants engaged in structured activity < 30 min per day twice a week Trained participants performed at least 1 hour of cycling or running 6 days per week over past 4 years
Combined Training Enhances Skeletal Muscle Mitochondrial Oxidative Capacity Independent of Age [14]	ET and CT significantly increased oxidative capacity and expression of mitochondrial proteins and transcription factors. CT induced the most robust improvements in mitochondria-related outcomes. Both ET and Ct consistently increased mitochondrial abundance. Increase in skeletal muscle mitochondrial OXPHOS, mRNA, and protein abundance of mitochondrial	ET: Cycling RT: Compound Lifts CT: Combination	34 young (18-30) and 31 old (>65)	ET performed cycling for 1 hour, 5 days/week for 8 weeks RT performed 4 sets of 8-10 reps targeting multiple muscle groups 4 days/week for 8 weeks CT cycled for 30 min 5 days/week and roughly 2/3 the RT volume 4 days/week ET: ~65% VO_2 CT: ~65% VO_2

	transcription factors and proteins. Most exercise training-induced improvements occurred independent of age ET: ↑ in PGC-1α 44% in young and 15% in old RT: ↑ 80% in young, 36% in old CT: ↑ 25% in young, 58% in old			
Exercise Increases mitochondrial PGC-1α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis [15]	There is no immediate increase in total PGC-1α content in skeletal muscle from END vs. SED, however, the protein content is significantly increased (2.4-fold) 3 h after acute endurance exercise.	Treadmill	3 month old mice: 12 sedentary, 12 forced-endurance, 12 forced endurance followed by 3 h recovery	15m/min. for 90 min.
A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms [16]	Protein content of PGC-1α was elevated ~24% post-training. Whole muscle PGC-1α was unchanged.	Cycling	7 healthy men (21 +/- 1 yr) recreationally active 2-3 times per week	6 sessions over 2 weeks 60s efforts of high-intensity cycling at peak oxygen uptake (V_{o2peak}) Intervals interspaced by 75s of low intensity cycling. 8 high-intensity intervals during first 2 training sessions, 10 intervals during next 2, and 12 intervals during final 2 sessions
Resistance exercise enhances the molecular signaling of mitochondrial biogenesis induced by endurance exercise in human skeletal muscle [17]	PGC-1α increased 10-fold in E subjects. PDK4 increased 14-fold. PGC-1α was 2x higher after ER than in E (1.9). PDK4 was 2.2-fold higher	Endurance Exercise: Cycling Resistance Exercise: Leg press	7 men, 2 women (26 +/- 1.2)	Each subject participated in two sessions: one with only endurance exercise (E) and the other with E followed by a bout of resistance exercise (ER) E and ER: Cycled at 60-70 rpm for 60 min ER subjects did same protocol followed by 15 min rest and then 6 sets of leg press with 3 min rest between sets. As many reps as possible. Cycling: 65% of VO2 max ER Leg Press: 70, 75, 80, 80,

				75, and 70% of the individual 1 RM.
Biochemical Adaptations in Muscle: Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle [18]	2-fold increase in the capacity to oxidize pyruvate. Activities of the enzymes of the mitochondrial electron transport chain approx. doubled. Concentration of cytochrome C increased by 2-fold. No evidence of swelling/gross alterations of mitochondria.	Treadmill	Male Wistar Rats 4 groups): Exercise Group Exercise Control Group Sedentary Control Group Sedentary Free Eating Group	Exercise Group: 8 degree incline, 4 days per week, 10 min 22m per min twice daily. Progressively increased over 12 weeks to 120 min at 31m per min with 12 sprints at 42m per min each lasting 30 sec Exercise Control: 10 min on treadmill 5 days per week, running speed increased to 31m per min over 6 weeks and maintained at this level
PGC-1 Isoforms and their target genes are expressed differently in human skeletal muscle following resistance and endurance training [19]	Both alternative promoter originated PGC-1α exon 1b- and 1b'-derived transcripts are strongly induced after EE and RE (170-fold after EE and 997-fold after RE) The proximal promoter originated PGC-1α exon 1a-derived transcripts are less inducible and were only upregulated after EE (1.7 fold)	Resistance Exercise Group (RE): Bilateral leg press Endurance Exercise Group (EE): Treadmill	RE: 11 healthy males EE: 8 Healthy males	RE: 10x10 leg press EE: 50 min. walk with 16.5 kg backpack Minutes 0-5 and 40-45: Speed=4.5 km/hr, incline=4 degrees Minutes 5-10 and 45-50: Speed=7 km/hr, slope=4.5km/hr Minutes 10-40: Speed adjusted to 75-85% HR max RE: Starting load=70% max
Exercise Training Increases Mitochondrial Biogenesis in the Brain [20]	SIRT1 mRNA expression was significantly increased (up to `2-fold) in the CX, FL, HY, HC, and MB, but not in the BS or CB. PGC-1α mRNA expression was significantly increased (up to ~3-fold) in the BS, CX, FL, HC, HY, and MB and there was a trend for an increase in the CB.	Treadmill	Male ICR mice. 16-19 exercise and sedentary	Eight weeks of treadmill running 1 hr/day, 6 days/week at 25 m/min. and 5% incline.
Exercise Induces Mitochondrial Biogenesis After Brain Ischemia in Rats [21]	PGC-1α protein levels were upregulated after 3 days of treadmill running, whereas 7 days of training was required for protein levels of NRF-1 and TFAM to increase above non-exercise controls	Treadmill	26 healthy male Sprague-Dawley rats 3 Groups: Ischemia: Sham control: Housed freely	Exercised on rat treadmill. 7 days at 30 min per day. Day 1-2: 5m/min. for 10 min., 9 m/min. for 10 min., 12 m/min. for last 10 min. 12 m/min on following 5 days. Middle cerebral artery was occluded.

			in cage, same exercise, no occlusion Non-exercise	
Exercise Training Attenuates Aging-Associated Mitochondrial Dysfunction in Rat Skeletal Muscle: Role of PGC-1α [22]	PGC-1α was 35% greater in old vs. young rats, but not difference was seen between OT and Y rats. Old rats showed a 2.3-fold increase in PGC-1α compared to sedentary (soleus muscle).	Treadmill	Male Fisher 344 x Brown Norway F_1 hybrid rats, 4 months (young) and 22 months (old)	Trained: motor-driven treadmill at 17.5 m/min, 10% grade for 45 min/day, 5 days/week for 12 weeks Sedentary: Walked 5 m/min for 15 min/day during the entire training period
Effect of High-Intensity Exercise on Aged Mouse Brain Mitochondria, Neurogenesis, and Inflammation [23]	PGC-1α and β levels were comparable between control and exercise group brains. Nuclear PGC-1α levels were 50% higher in exercise brains. Mitochondrial DNA copy number increased significantly	Six-lane treadmill	24 C57BL/6 male mice, 18 months old	Exercise: 8 weeks, 5 days per week, 2 sessions per day, six-lane treadmill, 5 degree incline. First week: 10 minute warm-up at 15 m/min followed by 30 minutes at 18 m/min Speed progressively increased the following 7 weeks: 21, 22, 23, 24, 25, 25, 26 m/min. Exercised above the lactate threshold.
Effect of Resistance Exercise Intensity on the Expression of PGC-1α Isoforms and the Anabolic and Catabolic Signaling Mediators, IGF-1 and Myostatin in Human Skeletal Muscle [24]	No statistically significant difference in total PGC-1α mRNA expression between trials	Lower body resistance exercise	Ten healthy men (23.7 +/- 2.8 years)	Two lower-body resistance exercise sessions of different intensities (50% of 1RM and 80% of 1RM) with equal volume load. Each exercise session was separated by 7 to 10 days.
Impact of Aerobic Exercise Training on Age-Related Changes in Insulin Sensitivity and Muscle Oxidative	There was an increase in genes involved in mitochondrial biogenesis (PGC-1α, 55%; NRF-1, 15%; TFAM, 85%)	Stationary bicycle	Forty-nine women and 41 men between the ages of 21-87 years	Stationary bicycle 3 sessions per week for 20 min at 70% max HR. Gradually increased so that the final month of training consisted of four sessions per week at 80% max HR for 40 min.

Capacity [25]				
Moderate Treadmill Exercise Ameliorates Amyloid-β-Induced Learning and Memory Impairment, Possibly via Increasing AMPK Activity and Up-Regulation of the PGC-1α /FNDC5/BDNF Pathway [26]	No significant difference in the protein levels of PGC-1α between the intact and sham groups, indicating that injection of DMSO had no effect on PGC-1α expression Aβ −injection suppressed the expression of PGC-1α The suppression of the PGC-1α pathway in the Aβ-treated rats is prevented by moderate treadmill exercise. Treadmill exercise significantly increased the mRNA levels of PGC-1α ($P < 0.05$) in the hippocampus of rats in Aβ-exe group compared to Abeta groups	Treadmill	70 adult male Winstar rats	5 days/week for 4 weeks First and second week: 30 min run at 10m/min Third week: 45 min per day (3 x 15 min sessions) at 15 m/min Fourth week: Ran 60 min per day (4 x 15 min sessions) at 15 m/min
Effect of Exercise on Mouse Liver and Brain Bioenergetics Infrastructure [27]	Liver PGC-1α was significantly increased but liver and brain PGC-1α protein levels were unchanged. Exercise appeared to induce at most a relatively selective mitochondrial biogenesis.	Treadmill	Twelve 4 month old C57BL/6 mice	Mice exercised for two sessions per day, five days per week for six weeks Three min warm up at 15 m/min plus 42 min at 18 m/min
Physical Exercise Improves Brain Cortex and Cerebellum Mitochondrial Bioenergetics and Alters Apoptotic, Dynamic, and Auto(mito)phagy markers [28]	PGC-1α and TFAM increased in the brain cortex in both exercise models (50% and 75%), while no alterations were detected in the cerebellum	Treadmill	18 male Sprague-Dawley rats	Rats exercised for 60 min/day starting at 18 m/min and progressively increasing to 30 m/min 5 days per week for 12 weeks
Long-Term Treadmill Exercise Induces Neuroprotective Molecular Changes in Rat Brain [29]	PGC-1α levels were higher in the cortex of exercise animals No hippocampal changes.	Treadmill	29 Sprague-Dawley rats	Rats exercised 4-5 days per week for 36 weeks Exercise started at 4.2 m/min and increased progressively by 1 m/min every 30m seconds to a speed of 12 m/min